

FIG. 1A

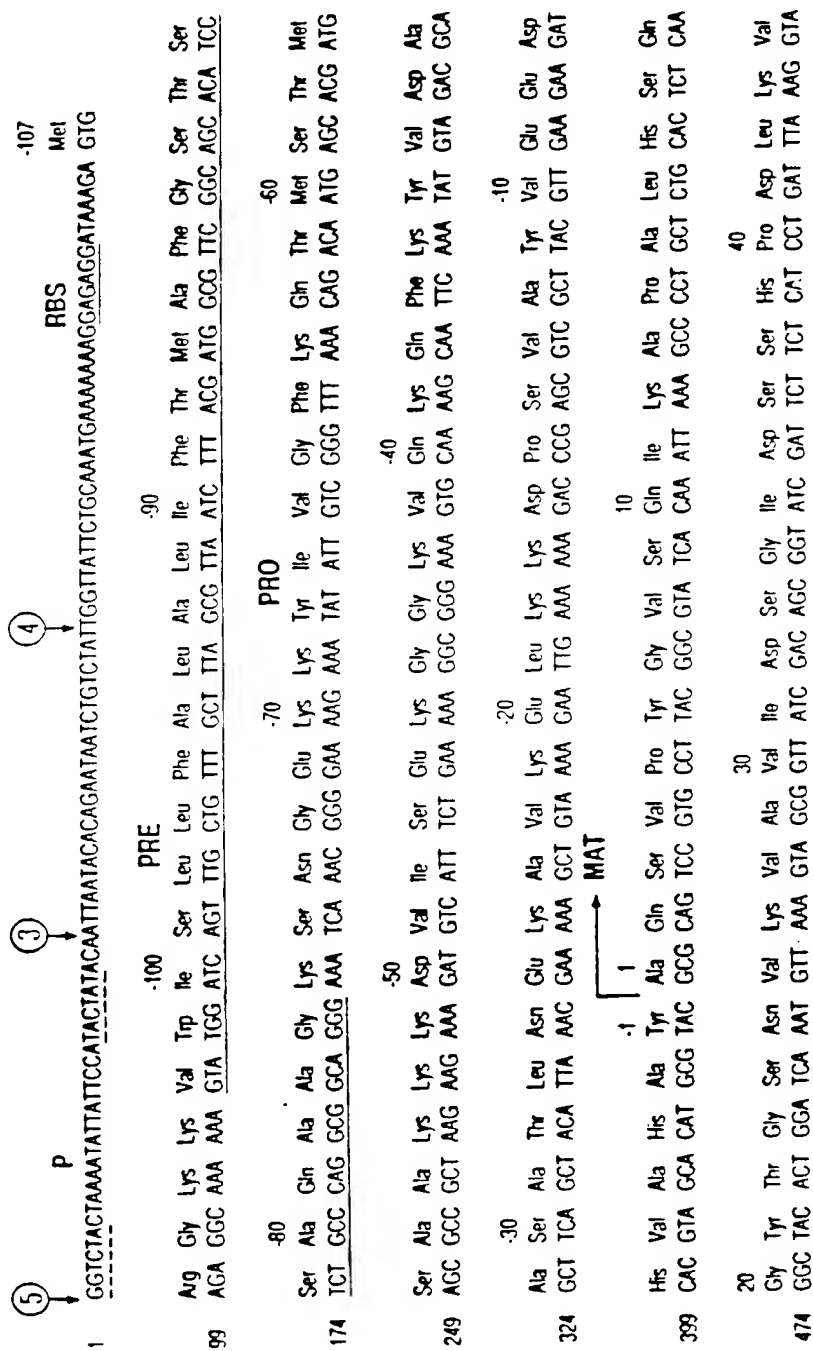


FIG. 1B-2

[illegible]

1316 ATAA TCGACGGATGGCTCCCTCTCGAAATTTTAAACGAGAAACGGGGTTGACCCGGCTCAGTCCCGTAAACGGCCAAAGTCTGAAACGTCTCAATCGCCG

1416 CTTC CCGGTTTCCGGTCAGCTCAATGCCGTAAACGGTCGGCGGGGTTTTCTGATACCGGAGACGGCATTCCGTAATCGGATC

FIG. 1B - 3

FIG. 1B-1

FIG._1B - 2

FIG. 1B - 3

FIG. 1B

CONSERVED RESIDUES IN SUBTILISINS FROM
BACILLUS AMYLOLIQUEFACIENS

1	10	20
A Q S V P . G	A P A . H . . .	G
21	30	40
. T G S . V K V A V . D . G		H P
41	50	60
D L . . . G G A S . V P		Q D
61	70	80
. N . H G T H V A G T . A A L N N S I		G
81	90	100
V L G V A P S A . L Y A V K V L G A .		G
101	110	120
S G . . S . L . . G . E W A . N		
121	130	140
V . N . S L G . P S . S		A . .
141	150	160
. G V . V V A A . G N . G . . .		
161	170	180
. Y P . . Y		A V G A .
181	190	200
D . . N . . A S P S . . G . . L D . .		A
201	210	220
P G V . . Q S T . P G . . Y		N G T
221	230	240
S M A . P H V A G A A A L		K . . .
241	250	260
W . . . Q . R . . L . N T		L G . .
261	270	
. . Y G . G L . N . . A A . .		

FIG._2

COMPARISON OF SUBTILISIN SEQUENCES FROM:

B. amyloliquefaciens

B. subtilis

B. licheniformis

B. lentus

01	10	20	30
A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P			
A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P			
A Q T V P Y G I P L I K A D K V Q A Q G F K G A N V K V A V L D T G I Q A S H P			
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T * H P			
41	50	60	70
D L K V A G G A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G			
D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T I A A L N N S I G			
D L N V V G G A S F V A G E A Y N * T D G N G H G T H V A G T V A A L D N T T G			
D L N I R G G A S F V P G E * P S T Q D D G N G H G T H V A G T I A A L N N S I G			
81	90	100	110
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D			
V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D			
V L G V A P S V S L Y A V K V L N S S G S G S Y S G I V S G I E W A T N G M D			
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N N G M H			
121	130	140	150
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V A A A A G N E G T S G			
V I N M S L G G P T G S T A L K T V V D K A V S S G I V V A A A A G N E G S S G			
V I N M S L G G A S G S T A M K Q A V D N A Y A R G V V V A A A A G N S G N S G			
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S			

FIG._3A

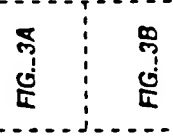
161
 S S S T V G G Y P P G K Y P P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A
 S T S T V G G Y P P A K Y P P S T I A V G A V N S S N Q R A S F S S A G S E L D V M A
 S T N T I G Y P A K Y D S V I A V G A V D S S N S N R A S F S S V G A E L E V M A
 * * * I S Y P A R Y A N A M A V G A T D Q N N N R A S F S S Q Y G A G L D I V A

201
 P G V S I Q S T L P G G N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N
 P G V S I Q S T L P G G T Y G A Y N G T S M A T P H V A G A A A L I L S K H P T
 P G A G V Y S T Y P T N T Y A T L N G T S M A S P H V A G A A A L I L S K H P N
 P G V N V Q S T Y P G S T Y A S L N G T S M A T P H V A G A A A L V K Q K N P S

241
 W T N T Q V R S S L E N T T K L G D S F Y Y G K G L I N V Q A A A Q
 W T N A Q V R D R L E S T A T Y L G N S F Y Y G K G L I N V Q A A A Q
 L S A S Q V R N R L S S T A T Y L G S S F Y Y G K G L I N V E A A A Q
 W S N V Q I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R

FIG._3B

FIG._3



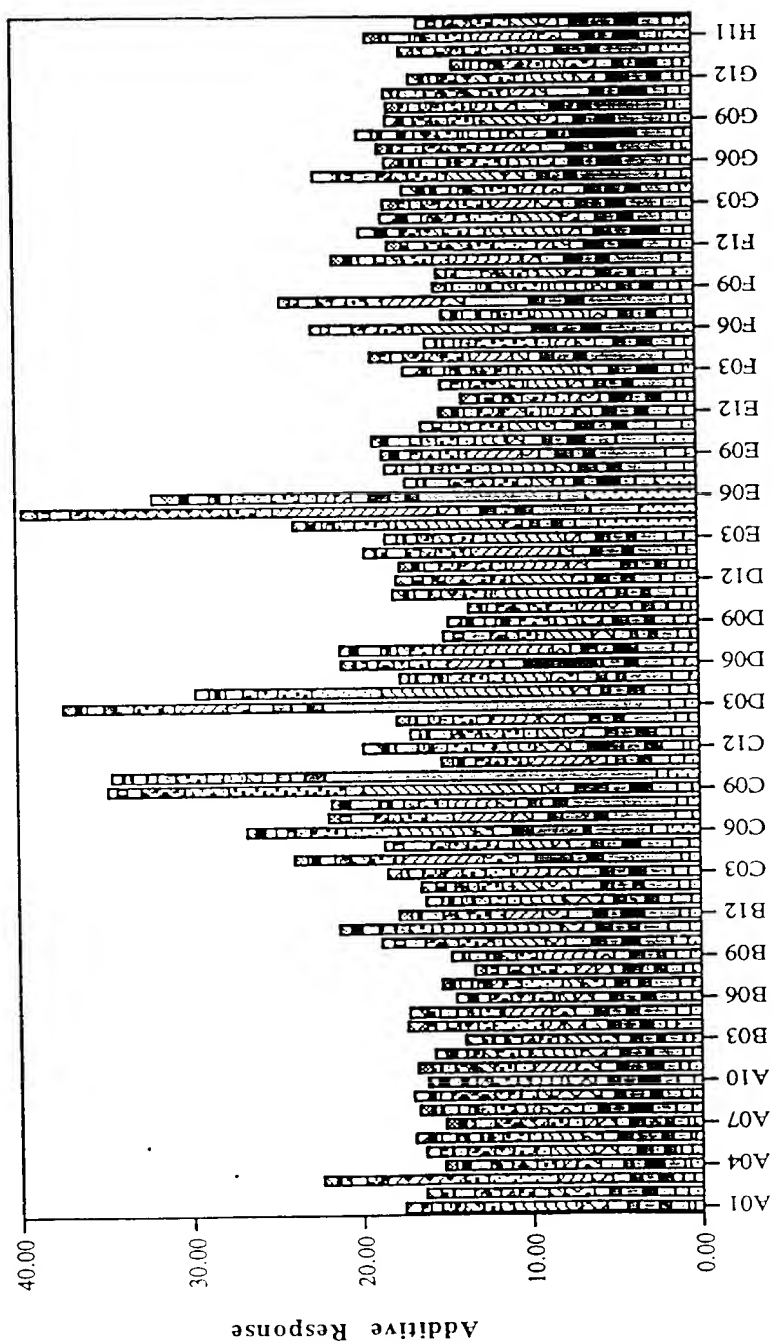


FIG. 4

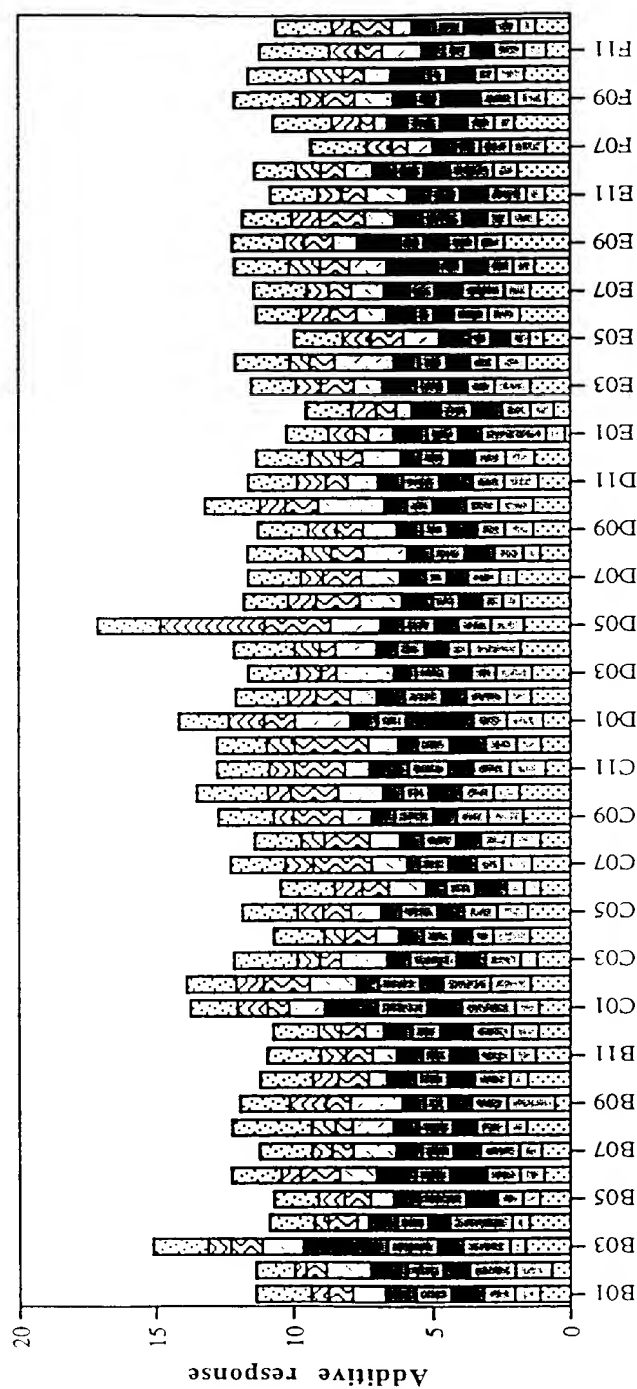


FIG. 5

1	A12	IKDFHVFRESRDAG	49	E12	SATSRGVLVVAASGN
2	A11	LEQAVNSATSRGVLV	50	E11	SRGVLVVAASGNSGA
3	A10	AQSVPWGISRVQAPA	51	E10	VLVVAASGNSGAGSI
4	A9	VPWGISRVQAPAAHN	52	E9	VAASGNSGAGSISYP
5	A8	GISRVQAPAAHNRGL	53	E8	SGNSGAGSISYPARY
6	A7	RVQAPAAHNRGLTGS	54	E7	SGAGSISYPARYANA
7	A6	APAAHNRGLTGSGVK	55	E6	GSISYPARYANAMAV
8	A5	AHNRGLTGSGVKVAV	56	E5	SYPARYANAMAVGAT
9	A4	RGLTGSGVKVAVLDT	57	E4	ARYANAMAVGATDQN
10	A3	TGSGVKVAVLDTGIS	58	E3	ANAMAVGATDQNNNR
11	A2	GVKVAVLDTGISTHP	59	E2	MAVGATDQNNNRASF
12	A1	VAVLDTGISTHPDLN	60	E1	GATDQNNNRASFQY
13	P12	LDTGISTHPDLNIRG	61	F12	DQNNNRASFQYGAG
14	B11	GISTHPDLNIRGGAS	62	F11	NNRASFSQYGAGLDI
15	B10	THPDLNIRGGASFVP	63	F10	ASFSQYGAGLDIVAP
16	B9	DLNIRGGASFVPGEF	64	F9	SQYGAGLDIVAPGVN
17	B8	IRGGASFVPGEFSTQ	65	F8	GAGLDIVAPGVNVQS
18	B7	GASFVPGEFSTQDGN	66	F7	LDIVAPGVNVQSTYP
19	B6	FVPGEFSTQDGNHGH	67	F6	VAPGVNVQSTYPGST
20	B5	GEPSTQDGNHGHGTHV	68	F5	GVNVQSTYPGSTYAS
21	B4	STQDGNHGHGTHVAGT	69	F4	VQSTYPGSTYASLNG
22	B3	DGNHGHGTHVAGTIAA	70	F3	TYPGSTYASLNGTSM
23	B2	GHGTHVAGTIAALNN	71	F2	GSTYASLNGTSMATP
24	B1	THVAGTIAALNNSIG	72	F1	YASLNGTSMATPHVA
25	C12	AGTIAALNNSIGVLG	73	G12	LNGTSMATPHVAGAA
26	C11	IAALNNSIGVLGVAP	74	G11	TSMATPHVAGAAALV
27	C10	LNNSIGVLGVAPSAE	75	G10	ATPHVAGAAALVKQK
28	C9	SIGVLGVAPSAELYA	76	G9	HVAGAAALVKQKNPS
29	C8	VLGVAPSAELYAVKV	77	G8	GAAALVKQKNPSWSN
30	C7	VAPSAELYAVKVLGA	78	G7	ALVKQKNPSWSNVQI
31	C6	SAELYAVKVLGASGS	79	G6	KQKNPSWSNVQIRNH
32	C5	LYAVKVLGASGSGSV	80	G5	NPSWSNVQIRNHLKN
33	C4	VKVLGASGSGSVSSI	81	G4	WSNVQIRNHLKNTAT
34	C3	LGASGSGSVSSIAQG	82	G3	VQIRNHLKNTATSLG
35	C2	SGSGSVSSIAQGLEW	83	G2	RNHLKNTATSLGSTN
36	C1	GSVSSIAQGLEWAGN	84	G1	LKNTATSLGSTNLYG
37	D12	SSIAQGLEWAGNNGM	85	H12	TATSLGSTNLYGSGL
38	D11	AQGLEWAGNNGMHVA	86	H11	SLGSTNLYGSGLVNA
39	D10	LEWAGNNGMHVANLS	87	H10	STNLYGSGLVNAEAA
40	D9	AGNNGMHVANLSLGS	88	H9	NLYGSGLVNAEAATR
41	D8	NGMHVANLSLGSPSP			
42	D7	HVANLSLGSPSPSAT			
43	D6	NLSLGSPSPSATLEQ			
44	D5	LGSPSPSATLEQAVN			
45	D4	PSPSATLEQAVNSAT			
46	D3	SATLEQAVNSATSRG			
47	D2	LEQAVNSATSRGVLV			
48	D1	AVNSATSRGVLVVA			

FIG. 6A

1	A12	IKDFHVYFRESRDAG	49	E12	KKIDVLNLSIGGPDF
2	A11	DAELHIFRVFTNNQV	50	E11	DVLNLSIGGPDFMDH
3	A10	PLRRASLSLGSGFWH	51	E10	NLSIGGPDFMDHPFV
4	A9	RASLSLGSGFWHATG	52	E9	IGGPDFMDHPFVDKV
5	A8	LSLGSGFWHATGRHS	53	E8	PDFMDHPFVDKVVWL
6	A7	GSGFWHATGRHSSRR	54	E7	MDHPFVDKVVWELTAN
7	A6	FWHATGRHSSRLLR	55	E6	PFVDKVVWELTANNVI
8	A5	ATGRHSSRLLRAIP	56	E5	DKVVWELTANNVIMVS
9	A4	RHSSRLLRAIPRQV	57	E4	WELTANNVIMVSAIG
10	A3	SRLLRAIPRQVAQT	58	E3	TANNVIMVSAIGNDG
11	A2	LLRAIPRQVAQTLQA	59	E2	NVIMVSAIGNDGPLY
12	A1	AIPRQVAQTLQADVL	60	E1	MVSAIGNDGPLYGTJ
13	B12	RQVAQTLQADVLWQM	61	F12	AIGNDGPLYGTLLNP
14	B11	AQTLQADVLWQMGYT	62	F11	NDGPLYGTLLNPNADQ
15	B10	LQADVLWQMGYTGAN	63	F10	PLYGTLLNPNADQMDV
16	B9	DVLWQMGYTGANVRV	64	F9	GTLLNPNADQMDVIGV
17	B8	WQMGYTGANVRVAVF	65	F8	NNPADQMDVIGVGGI
18	B7	GYTGANVRVAVFDTG	66	F7	ADQMDVIGVGGIDFE
19	B6	GANVRVAVFDTGLSE	67	F6	MDVIGVGGIDFEDNI
20	B5	VRVAVFDTGLSEKHP	68	F5	IGVGGIDFEDNIARF
21	B4	AVFDTGLSEKHPHFK	69	F4	GGIDFEDNIARFSSR
22	B3	DTGLSEKHPHFKNVK	70	F3	DFEDNIARFSSRGMT
23	B2	LSEKHPHFKNVKERT	71	F2	DNIARFSSRGMTTWE
24	B1	KHPHFKNVKERTNWT	72	F1	ARFSSRGMTTWELPG
25	C12	HFKNVKERTNWTNER	73	G12	SSRGMTTWELPGGYG
26	C11	NVKERTNWTNERTLD	74	G11	GMTTWELPGGYGRMK
27	C10	ERTNWTNERTLDDGL	75	G10	TWELPGGYGRMKPDI
28	C9	NWTNERTLDDGLGHG	76	G9	LPGGYGRMKPDIVTY
29	C8	NERTLDDGLGHGTFV	77	G8	GYGRMKPDIVTYGAG
30	C7	TLDDGLGHGTFVAGV	78	G7	RMKPDIVTYGAGVRG
31	C6	DGLGHGTFVAGVIAS	79	G6	PDIVTYGAGVRGSGV
32	C5	GHGTFVAGVIASMRE	80	G5	VTYGAGVRGSGVKGG
33	C4	TFVAGVIASMRECQG	81	G4	GAGVRGSGVKGGCRA
34	C3	AGVIASMRECQGFAP	82	G3	VRGSGVKGGCRALSG
35	C2	IASMRECQGFAPDAE	83	G2	SGVKGGCRALSGTSV
36	C1	MRECQGFAPDAELHI	84	G1	KGGCRALSGTSVASP
37	D12	CQGFAPDAELHIFRV	85	H12	CRALSGTSVASPVVA
38	D11	FAPDAELHIFRVFTN	86	H11	LSGTSVASPVVAGAV
39	D10	DAELHIFRVFTNNQV	87	H10	TSVASPVVAGAVTLL
40	D9	LHIFRVFTNNQVSYT	88	H9	ASPVVAGAVTLLVST
41	D8	FRVFTNNQVSYTSWF	89	H8	VVAGAVTLLVSTVQK
42	D7	FTNNQVSYTSWFLDA	90	H7	GAVTLLVSTVQKREL
43	D6	NQVSYTSWFLDAFNY	91	H6	TLLVSTVQKRELVNP
44	D5	SYTSWFLDAFNYAIL	92	H5	VSTVQKRELVNPASM
45	D4	SWFLDAFNYAILKKI	93	H4	VQKRELVNPASMKQA
46	D3	LDAFNYAILKKIDVL	94	H3	RELVNPASMKQALIA
47	D2	FNYAILKKIDVLNLS	95	H2	VNPASMKQALIASAR
48	D1	AILKKIDVLNLSIGG	96	H1	ASMKQALIASARRLP

FIG. 6B

97	I12	IKDFHVYFRESRDAG
98	I11	DAELHIFRVFTNNQV
99	I10	KQALIASARRLPGVN
100	I9	LIASARRLPGVNMFE
101	I8	SARRLPGVNMFEQGH
102	I7	RLPGVNMFEQGHGKL
103	I6	GVNMFEQGHGKLDLL
104	I5	MFEQGHGKLDLLRAY
105	I4	QGHGKLDLLRAYQIL
106	I3	GKLDLLRAYQILNSY
107	I2	DLLRAYQILNSYKPQ
108	I1	RAYQILNSYKPQASL
109	J12	QILNSYKPQASLSPS
110	J11	NSYKPQASLSPSYID
111	J10	KPQASLSPSYIDLTE
112	J9	ASLSPSYIDLTECPY
113	J8	SPSYIDLTECPYMW
114	J7	YIDLTECPYMWPYCS
115	J6	LTECPYMWPYCSQPI
116	J5	CPYMWPYCSQPIYYG

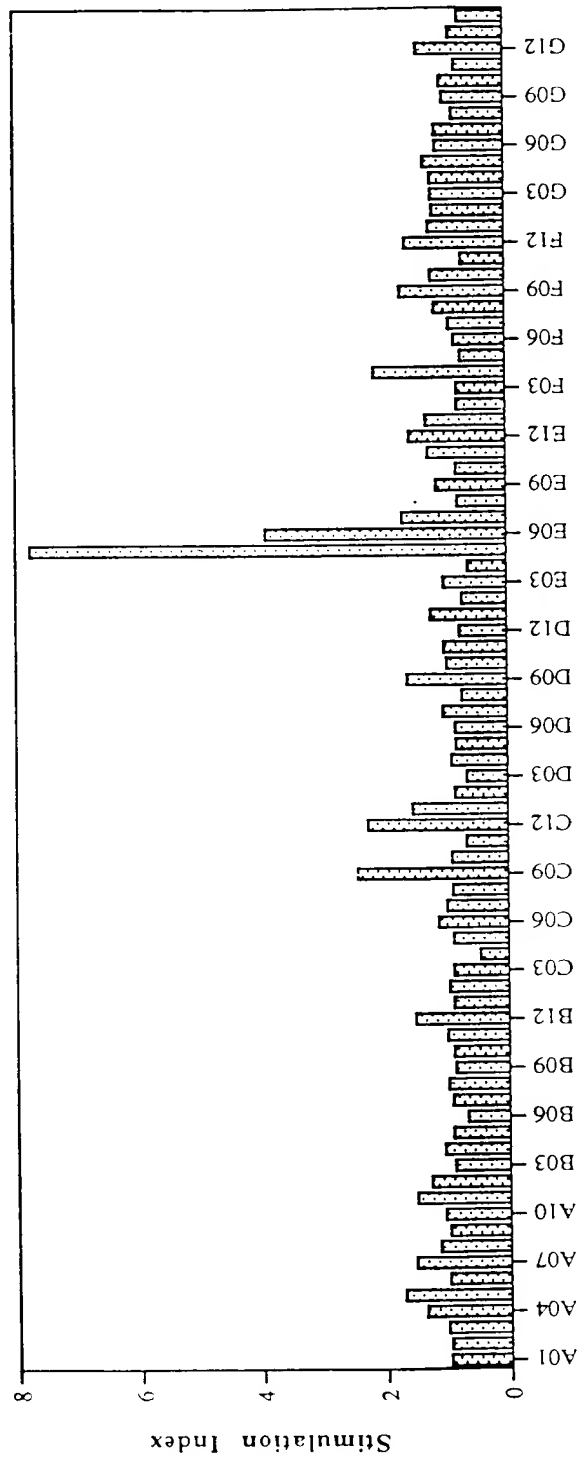
FIG. 6C

MKLVNIWLLLLLVLLCGKKHLGDRLEKKSFEKAPCPGCSHLTLKVEFSSTVVEYEYIVAFNGYFT
AKARNSFISSALKSSEVDNWRIIPRNNPSSDYPSEFEVIQIKEKQKAGLLTLEDHPNIKRVTPQR
KVFRSLKYAESDPTVPCNETRWSQKWQSSRPLRRASLSLGS GFWHATGRHSSRLLRAIPRQVAQ
TLQADVLWQMGYTGANVRVAVFDTGLSEKHPHFKNVKERTNWTNERTLDDGLGHGTFVAGVIAS
RECQGFAPDAELHIFRVFTNNQVSYTSWFLDAFNAILKKIDVLNLSIGGPDFMDHPFVDKVWEL
TANNVIMVSAIGNDGPLYGTLLNNPADQMDVIGVGGIDFEDNIARFSSRGMTTWELPGGYGRMKPD
IVTYGAGVRGSGVKGGCRALSGTSVASPVVAGAVTLLVSTVQKRELVPASMKQALIASARRLP
VNMFEQGHGKLDLLRAYQILNSYKPQASLSPSYIDLTECPYMWPYCSQPIYYGGMPTVVNVITILN
GMGVTGRIVDKPDWQPYLPQNGDNIEVAFSYSSVLWPWSGYLAISISVTKKAASWEGIAQGHVMI
TVASPAETESKNGAEQTSTVKLPIKVKIIPTPPRSKRVLWDQYHNLRYPPGYFPRDNLRMKNDPL
DWNGDHIHTNFRDMYQHLRSMGYFVEVLGAPFTCFDASQYGTLLMVDSEEEYFPEEIAKLRRD
NGLSLVIFSDWYNTSVMRKVKFYDENTRQWWMPDTGGANIPALNELLSVWNMGFSDGLYEGETL
ANHDMYYASGCSIAKFPEDGVVITQTFKDQGLEVLKQETAVVENVPILGLYQIPAEAGGGRIVLYG
DSNCLDDSHRQKDCFWLLDALLQYTSYGVTPPSLSHSGNRQRPPSGAGSVTPERMEGNHLHRYSK
VLEAHLGDPKPRPLPACPRLSWAKPQPLNETAPSNLWKHQKLLSIDLDKVVLNFRSNRPQVRPL
SPGESGAWDIPGGIMPGRYNQEVGQTI PVFAFLGAMVVLAFVQINKAKSRPKRRKPRVKRPQL
MQQVHPPKTPSV

FIG. 7

	10	20	30	40	50	
BPN'	AQSVPYGVSQ- IKAPALHSQGYTGSNVKVAVIDSGIDSSHDPDLK-VAGGA					48
SAVINASE	AQSVPWGISR-VQAPAAHNRGLTGSGVKVAVLDTGI-STHPDLN-IRGGA					47
S2HSBT	-RAIPRQVAQTLQADVLWQMGYTGANVRVAVFDTGLSEKHPHFKNVKERT					49
	60	70	80	90	100	
BPN'	SMVPSETNPFQDNNSHGTHVAGTVAALNNSIGVLGVAPSASLYAVKVLGA					98
SAVINASE	SFVPGEPST-QDGNHGHGTHVAGTIAALNNSIGVLGVAPSALYAVKVLGA					96
S2HSBT	NW--TNERTLDDGLGHGTFVAGVIAASMRECQGF---APDAELHIFRVFTN					94
	110	120	130	140	150	
BPN'	DGSGQYSWIINGIEWAIAANNMDVINMSLGGPS-GSAALKAAVDKAVASGV					147
SAVINASE	SGSGSVSSIAQGLEWAGNNGMHVANLSLGSPS-PSATLEQAVNSATSRGV					145
S2HSBT	NQVSYTSWFLDAFNIAILKKIDVLNLSIGGPDFMDHPFVDKVVWELTANNV					144
	160	170	180	190	200	
BPN'	VVVAAAGNEGTSGSSSTVGYPGKYPSVIAVGAVDSSNQRASFSVSGPEL-					197
SAVINASE	LVVAASGNSGA-----GSISYPARYANAMAVGATDQNNNRASFQYAGAL-					191
S2HSBT	IMVSAIGNDGP--LYGTLLNNPADQMDVIGVGGIDFEDNIARFSSRGMTTW					192
	210	220	230	240	250	
BPN'	-----DVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALIL					235
SAVINASE	-----DIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVK					229
S2HSBT	ELPGGYGRMKPDIVTYGAGVRGSGVKGGCRALSGTSVASPVVAGAVTLLV					242
	260	270	280	290		
BPN'	SKHPNWTNTQ---VRSSLENTTTKLGDSTFYYGKGLINVQAAAQ				275	
SAVINASE	QKNPSWSNVQ---IRNHLKNTATSLGSTNLYGSGLVNAEAATR				269	
S2HSBT	STVQKRELVPNPA SMKQALIASARRLPGVNMFEEQG-----HGKL				280	

FIG. 8



Well Position

FIG. 9

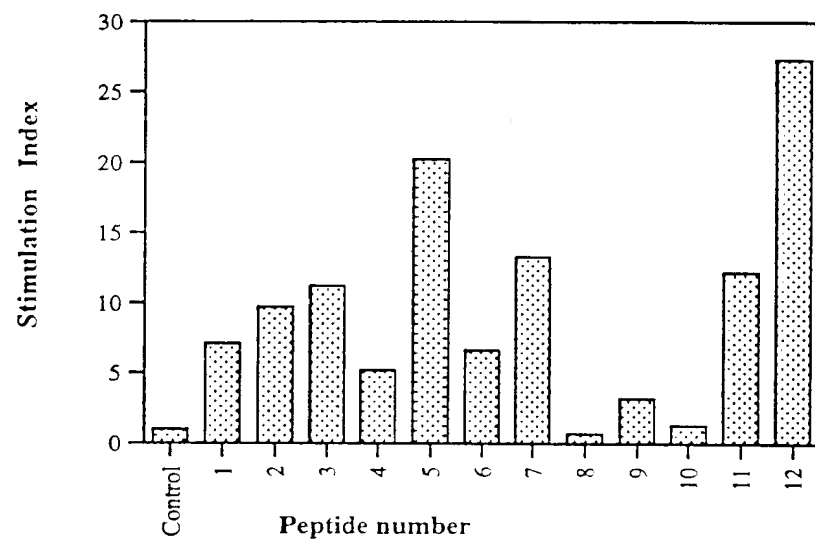
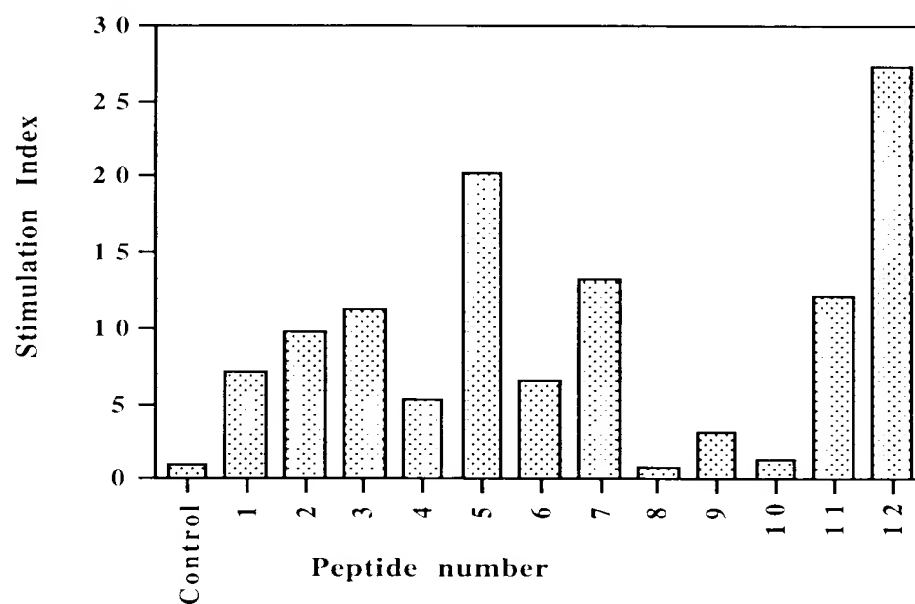


FIG. 10



Peptide number	Sequence
1 (unmodified sequence)	GSISYPARYANAMAV
2	ASISYPARYANAMAV
3	GAISYPARYANAMAV
4	GSASYPARYANAMAV
5	GSIAYPARYANAMAV
6	GSISAPARYANAMAV
7	GSISYAARYANAMAV
8	GSISYPAAAYANAMAV
9	GSISYPARAANAMAV
10	GSISYPARYAAAMAV
11	GSISYPARYANAAAV
12	GSISYPARYANAMAA

FIG. 11

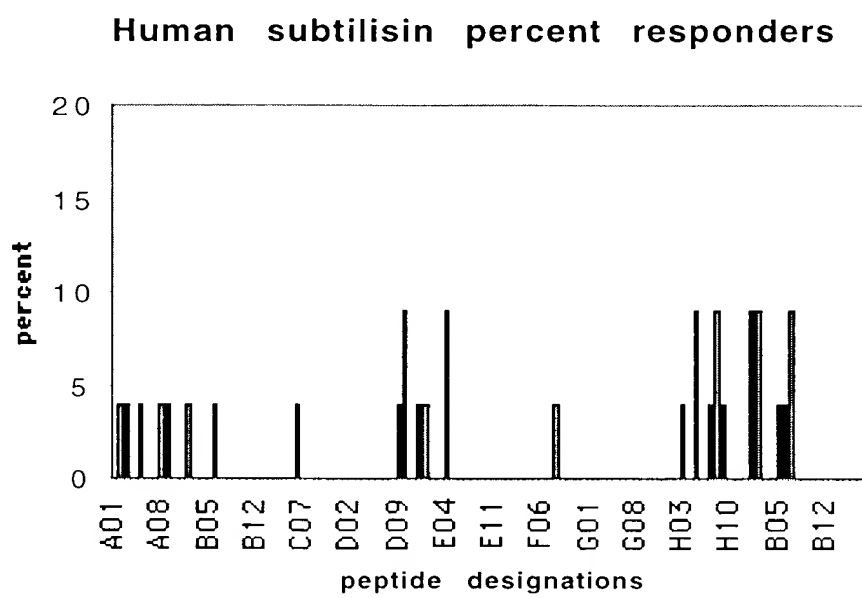


FIG. 12

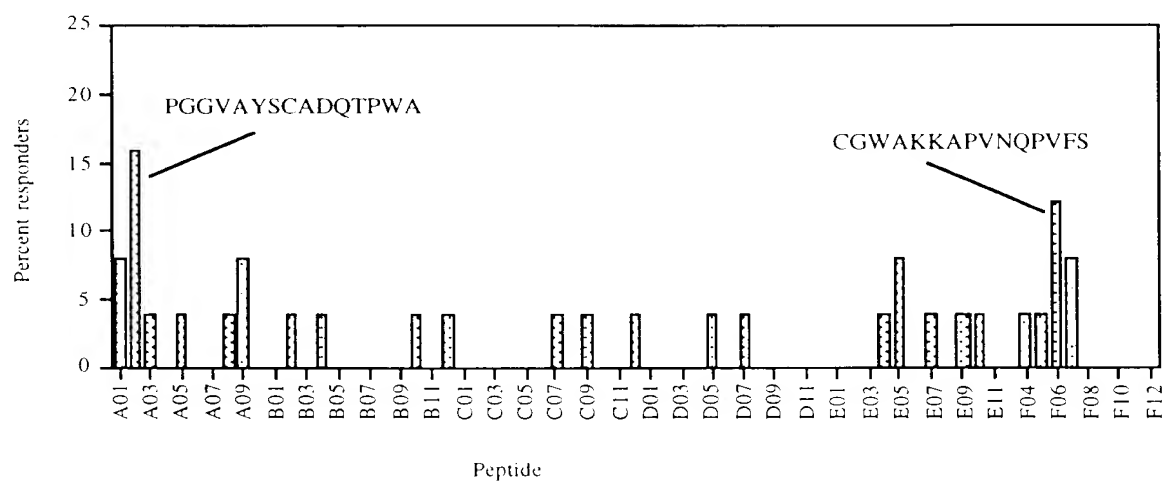


FIG. 13A

1	2	3	4	5
1234567890	1234567890	1234567890	1234567890	1234567890
MRSSPLLPSA	VVAALPVLAL	AADGRSTRYW	DCCKPS <u>CGWA</u>	<u>KKAPVNQPVF</u>
<u>SCNANFQRIT</u>	DFDAKSGCEP	<u>GGVAYSCADQ</u>	<u>TPWAVNDDFA</u>	LGFAATSIAG
SNEAGWCCAC	YELTFTSGPV	AGKKMVVQST	STGGDLGSNH	FDLNIPGGGV
GIFDGCTPQF	GGLPGQRYGG	ISSRNECDRF	PDALKPGCYW	RFDWFKNADN
PSFSFRQVQC	PAELVARTGC	RRNDDGNFPA	VQIPSSSTSS	PVNQPTSTST
TSTSTTSSPP	VQPTTPSGCT	AERWAQ		

FIG. 13B

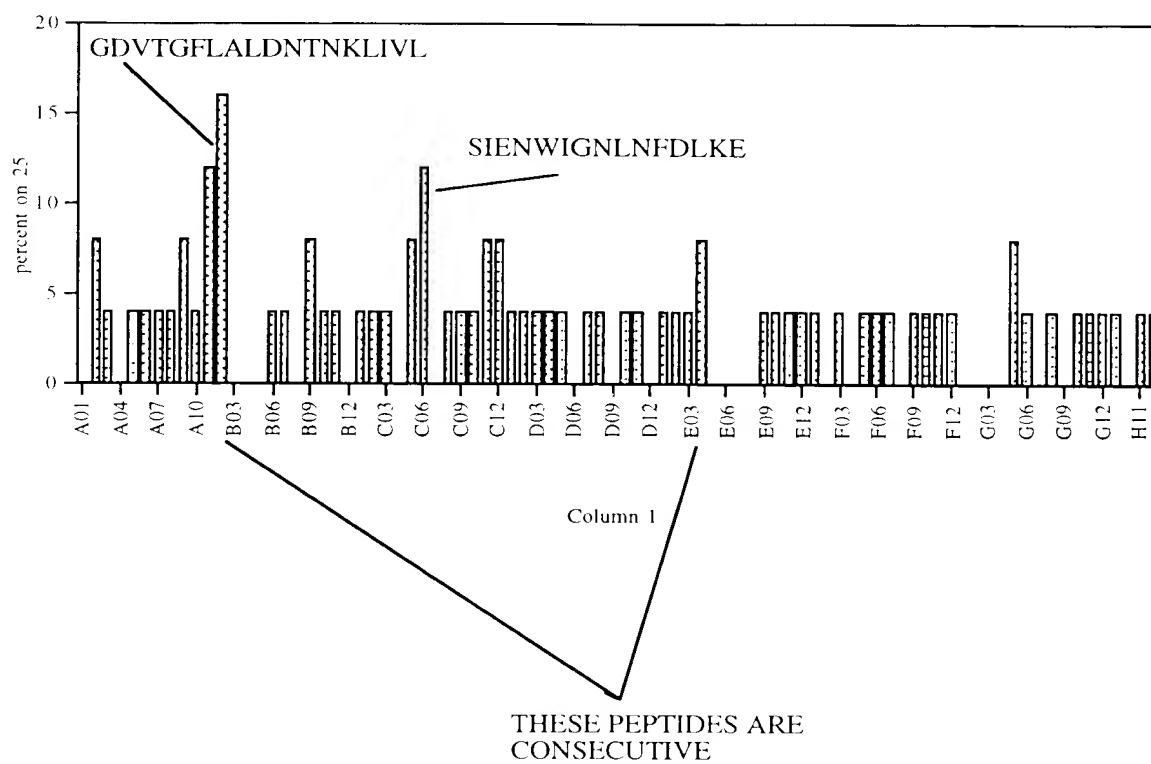


FIG. 14A

1 mrsslvffv sawtalaspi rrevsqdlfn qfnlfaqysa aaycgknnda
51 pagtnitctg nacpevekad atflysfeds gvgdvtgfla ldntnklivl
101 sfrgsrsien wignlnfdlk eindicsgcr ghdgftsswr svadtlrqkv
151 edavrehpdy rvvftghslg galatvagad lrgngydidv fsygaprvgn
201 rafaefltvq tggtyrith tndivprlpp refgyshssp eywiksgtlv
251 pvtrndivki egidatggnn qpnipdipah lwyfgligtc 1

FIG. 14B

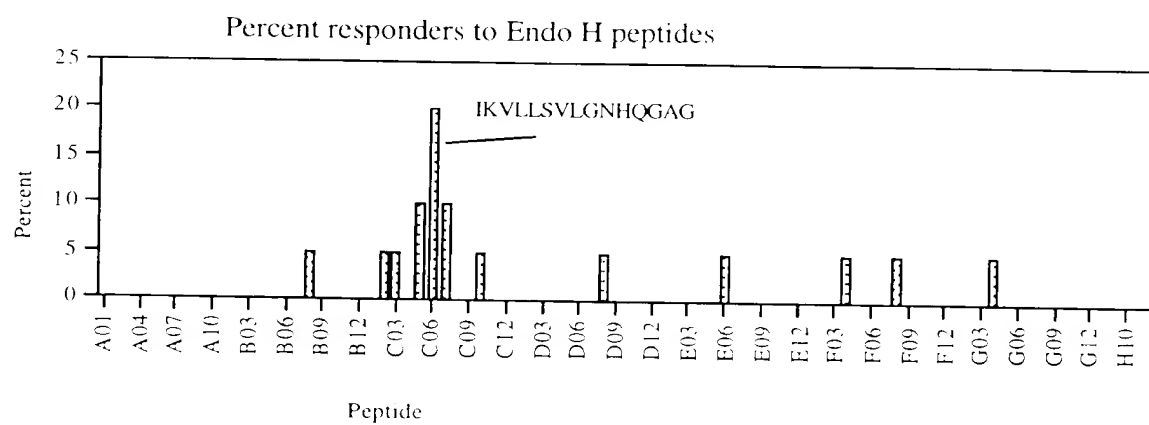


FIG. 15A

1 mftpvrerrvr taalalsaaa alvlgstaas gasatpspap apapapvkqg
51 ptsvayvevn nnsmlnvgky tladgggnaf dvavifaani nydtggtkay
101 lhfneenvgrv ldnavtqirp lqqggikvll svlgnhggag fanfpsqqaa
151 safakqlsda vakygldgvd fddeyaeygn ngtaqpndss fvhltalra
201 nmpdkiiisly nigpaasrls yggvdvsdkf dyawnpyygt wqvpgialpk
251 aqlspaavei grtsrstvad larrtvdegy gvyltynldg gdrtadvsaf
301 trelygseav rtp

FIG. 15B

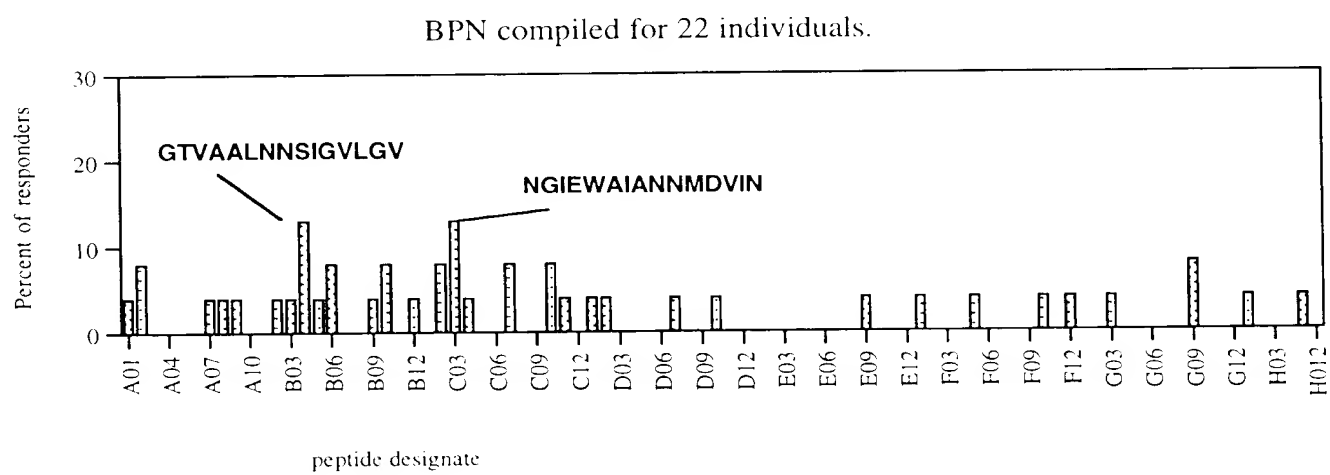


FIG. 16

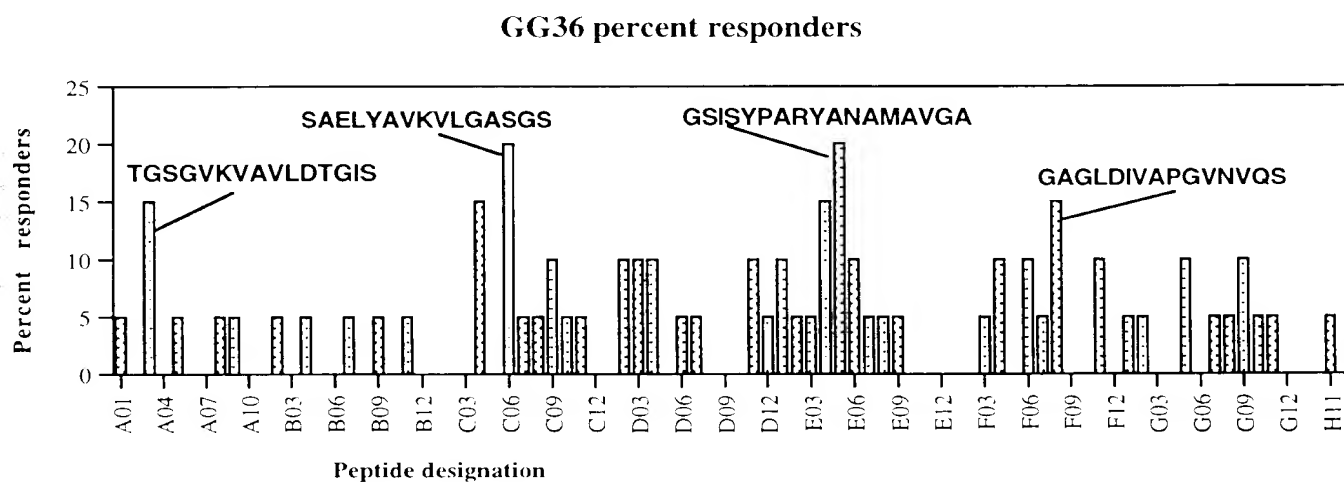


FIG. 17

Hybrid enzyme sequence (GG36-BPN)

GG36

AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGISTHPDLNIRGGASFVPGEPTQDGNGH

BPN

GTHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQGLEWAGNNGMHVINMSLGG

Δ

GSAALKAAVDKAVASGVVVVAAAGNEGTSAGSSSTVGYPGKYPSVIAVGAVDSSNQRASFSSVGP

ELDVMAPGVSIQSTLPGNKYGAYNGTSMASPHVAGAAALILSKHPNWTNTQVRSSLENTTTKLGD

SFYY GKGLINVQAAAQ

FIG. 18